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# Short term changes in the expression of lipogenic genes in broilers (*Gallus gallus*) ☆

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**Abstract**

The purpose of these experiments were to determine possible relationships between certain indices of lipid metabolism and specific gene expression in chickens fed graded levels of dietary crude protein. Male, broiler chickens growing from 7 to 28 days of age were fed diets containing 12 or 30% protein ad libitum. Both groups were then switched on day 28 to the diets containing the opposite level of protein. Birds were killed on day 28 (basal values prior to the switch) and at 12, 18 and 24 h post switch. Measurements taken included in vitro lipogenesis, malic enzyme activity the expression of the genes for malic enzyme, fatty acid synthase and acetyl coenzyme carboxylase. In vitro lipogenesis and malic enzyme activity were inversely related to dietary protein levels (12 to 30%) and to acute changes from 12 to 30%. Malic enzyme, fatty acid synthase and acetyl coenzyme A carboxylase genes were constant over a dietary protein range of 12 to 21% as in previous experiments, but decreased by feeding a 30% protein diet in the present experiments (acute or chronic feeding). Results of the present study demonstrate a continued role for protein in the regulation of broiler metabolism. Metabolic regulation at the gene level only occurs when feeding very high levels of dietary protein.
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*Keywords:* Lipogenic genes; Broilers; Chicken

## 1. Introduction

Over a period of several years, we have studied the effects of dietary protein levels on various indices of lipid metabolism. Briefly, increased dietary protein decreases de novo lipogenesis and the activities of several associated enzymes. In contrast, increased dietary protein increases plasma thyroxine (T4) and insulin-like growth factor I (IGF-I) while decreasing plasma triiodothyronine (T3) and growth hormone (GH). Likewise, acute changes in dietary protein whether changing from a high to a low protein diet or vice versa, will cause a rapid increase or decrease, respectively, in de novo lipogenesis.

Carbohydrates, fats and protein participate along with the endocrine system in changes in gene expression elicited by nutrients (Clark and Abraham, 1992). It may be possible to regulate gene expression anywhere from transcription to the actual enzyme protein. It would seem more efficient for regulation to occur at steps down stream from the actual transcription process. It should be emphasized that nutritional factors could regulate enzyme activity by any combination of factors impacting translation and post-translational events.

Acetyl CoA, a primary ingredient in the initial phase of fat synthesis, is formed by the reaction catalyzed by ATP citrate lyase. It is somewhat debatable if this step can be rate limiting for the de novo synthesis of fat. There is no argument, however, concerning acetyl CoA's control of de novo lipogenesis. The carboxylation of acetyl CoA produces malonyl CoA which condenses with acetyl CoA or fatty acyl CoA under the control of fatty acid synthase. The

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latter step requires NADPH that is provide by the pentose cycle in mammals. In contrast, avian lipogenesis depends upon NADPH provided by a reaction catalyzed by malic enzyme due to a deficiency in pentose cycle activity. We chose to analyze malic enzyme activity and gene expression because of its previously mentioned central role in providing reducing equivalents to support de novo lipogenesis. In our experience, measurements of in vitro lipogenesis approximate the rate limiting activities of both acetyl CoA carboxylase and fatty acid synthase.

The purposes of this experiment were to further study the metabolic effects of changes in the levels of dietary protein treatments and to determine if changes in mRNA for certain lipogenic enzymes related to changes in metabolic rates noted with various levels of dietary crude protein.

## 2. Materials and methods

### 2.1. Animals and diets

All chickens (*Gallus gallus*) were held under a quarantine that was certified by the station veterinarian. Chickens were observed daily for healthiness. One authorized animal caretaker was assigned to maintain chickens over the course of the experiments. In addition, the research protocols were approved by the Beltsville Agricultural Research Animal Care Committee. Birds were allowed to consume both feed and water on an ad libitum basis. Birds were kept in electrically heated battery-brooders (4 birds/pen replicate) in an environmentally controlled room (22 °C). A 12-h light (0600 to 1800 h), 12-h dark (1800 to 0600 h) cycle was maintained.

Birds were fed a standard starter diet (21% protein) until 7 days of age. The birds were then fed diets containing either 12 or 30% protein for 21 days (Table 1). The calculated carbohydrate content of each of the diets was determined by using published values for the protein and fat contents of each of the dietary ingredients, multiplying by 16.7 J for protein and 37.6 J for fat and subtracting those figures from the total metabolizable energy of the ingredient. It was assumed that this remainder was the carbohydrate contribution to the energetic value of that ingredient. The dietary treatments were then reversed. Birds were then selected (0, 12, 18 and 24 h post reversal) and killed by decapitation.

### 2.2. In vitro techniques — lipogenesis

Livers were excised and then sliced (MacIlwain Tissue Chopper; 0.3 mm). Quadruplicate explants were incubated at 37 °C for 2 h in Hanks' balanced salts (Hanks and Wallace, 1949; Rosebrough and Steele, 1988) containing 10 mM-HEPES and 10 mM Na-[2-$^{14}$C]acetate (166 MBq/mol). All incubations were conducted in 3 mL volumes at 37 °C for 2 h under a 95% $O_2$-5% $CO_2$ atmosphere. At the end of the stated incubation periods, the explants were placed in 10 mL of 2:1 chloroform: methanol for 18 h according to Folch et al. (1957). The extracts were evaporated to dryness and dispersed in scintillation fluid. Radioactivity in the extracts was measured by liquid scintillation spectroscopy. In vitro lipogenesis was expressed as μmoles of acetate incorporated into lipids per g of tissue.

Table 1
Composition of diets

| | Dietary protein, % | |
|---|---|---|
| | 12 | 30 |
| *Ingredient* | | |
| Isolated soy protein[a] (g/kg) | | 100 |
| Soybean meal (g/kg) | 112 | 400 |
| Corn meal (g/kg) | 767 | 400 |
| Corn oil (g/kg) | 17 | 40 |
| Sand (g/kg) | 15 | |
| Dicalcium phosphate (g/kg) | 40 | 40 |
| Limestone (g/kg) | 10 | 10 |
| Sodium chloride (g/kg) | 3 | 3 |
| L-methionine[b] (g/kg) | | 5 |
| Selenium premix[c] (g/kg) | 1 | 1 |
| Mineral premix[d] (g/kg) | 1 | 1 |
| Vitamin premix[e] (g/kg) | 5 | 5 |
| Cellulose (g/kg) | 30 | |
| *Calculated composition* | | |
| Metabolizable energy (MJ/kg) | 12.8 | 12.8 |
| Lysine (g/kg) | 6.0 | 17.3 |
| Sulfur amino acids (g/kg) | 10.3 | 10.3 |

[a] Soya-bean protein grade II (900 g/kg crude protein, 21726); Nutritional Biochemicals, PO Box 22400, Cleveland, Ohio 44122, USA.
[b] L-methionine (18915), US Biochemicals, PO Box 22400, Cleveland, Ohio 44122, USA.
[c] Provided 0.2 mg Se/kg of diet.
[d] Provided (mg/kg of diet): manganese 100, iron 100, copper 10, cobalt 1, iodine 1, zinc 100 and calcium 89.
[e] Provided (mg/kg of diet): retinol 3.6, cholecalciferol 0.075, biotin 1, vitamin E 10, riboflavin 10, pantothenic acid 20, choline 2 g, niacin 100, thiamine 10, vitamin $B_6$ 10, menadione sodium bisulfite 1.5, cyanocobalamin 0.1, folic acid 2 and ethoxyquin 150.

### 2.3. In vitro techniques — enzyme assays

Remaining liver tissues were homogenized (1:10, wt./vol.) in 100 mM-imidazole-250 mM sucrose (pH 7.5)-3.3 mM-β-mercaptoethanol and centrifuged at 12,000 ×*g* for 30 min. The supernatant fractions were kept at −80 °C until analyzed for the activities of malic enzyme (EC 1.1.1.40), isocitrate dehydrogenase (EC 1.1.1.42) and aspartate aminotransferase (EC 2.6.1.1).

Malic enzyme activity was determined by a modification of the method of Hsu and Lardy (1969). Reactions contained 50 mM HEPES (pH 7.5), 1 mM NADP, 10 mM $MgSO_4$ and the substrate, 2.2 mM L-malate (disodium salt) in a total volume of 1 mL. Portions (50 μL) of the 12,000 *g* supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 °C.

Isocitrate: NADP+ oxidoreductase-[decarboxylating] activity was determined by a modification of the method of Cleland et al. (1969). Reactions contained 50 mM HEPES (pH 7.5), 1 mM NADP, 10 mM $MgCl_2$ and the substrate, 4.4 mM DL-isocitrate in a total volume of 1 mL. Portions (50 μL) of the 12,000 ×*g* supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 30 °C.

Aspartate aminotransferase activity was determined by a modification of the method of Martin and Herbein (1976).

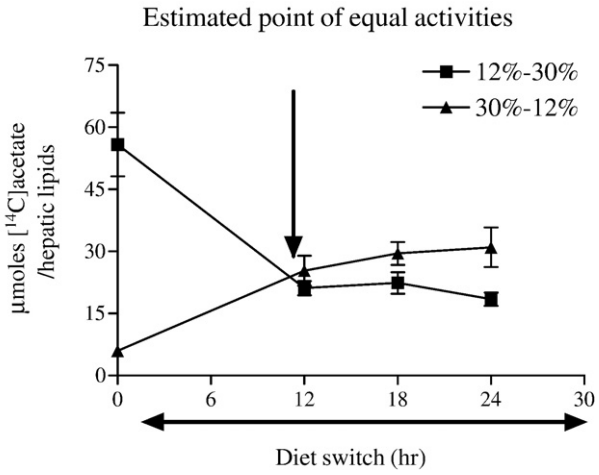


Fig. 1. In vitro lipogenesis in chickens switched from a low to a high protein or high to low protein diet. Chickens were initially offered diets containing either 12 or 30% crude protein from 7 to 28 day. Dietary treatments for half the chickens were then reversed and birds were incrementally sampled from 0 to 24 h post reversal. The first number in each legend denotes the dietary crude protein for the 7 to 28-day period and the second number denotes the diet during the reversal period. Values for in vitro lipogenesis (IVL) are expressed as µmol of [2-$^{14}$C]acetate incorporated/g of liver into lipids. Data are means of four chickens at each time point. Bars represent SEM.

Reactions contained 50 mM HEPES, 200 mM L-aspartate, 0.2 mM NADH, 1000 units per liter malate: NAD+ oxidoreductase (EC 1.1.1.37) and the substrate, 15 mM α ketoglutarate in a total volume of 1.025 mL. Portions (25 µL) of the 12,000 ×*g* supernatants (diluted 1:20) were preincubated in the presence of the first four ingredients. Reactions were initiated by adding the substrate and following the rate of oxidation of NADH at 340 nm at 30 °C. Enzyme activities are expressed as µmoles of product formed/min under the assay conditions (Rosebrough and Steele, 1985a,b).

### 2.4. In vitro techniques — lipogenic enzyme gene expression

Total RNA was isolated from 100 mg of liver using the Tri-Reagent procedure (Life Technologies, Rockville, MD, USA) measured spectrophotometrically and qualitatively by agarose gel electrophoresis (migration of 18 and 28s RNA). RT-PCR reactions (25 µL) consisted: of 1–2 µg total RNA, 1× QuantiTect SYBR Green RT-PCR Master Mix, variable amounts of RNase water, QuantiTect RT mix, 2.5 mM Mg and 0.5 µM each of each gene specific primer including a set for β-actin, the internal standard. Reverse transcription proceeded for 30 min at 50 °C in the presence of both Omniscript and Sensiscript. Initially, a 15 min incubation at 95 °C was used to inactivate the RT reaction and to activate the HotStarTaq DNA Polymerase. The following comprised 40 PCR cycles: Denaturation for 30 s at 94 °C, followed by annealing for 30 s at 58 °C and extension for 60 s at 72 °C. Fluorescence data was collected in the latter stage by noting SYBR Green incorporation into the extended DNA. RT-PCR produced dsDNA amplicons of 423, 200, 447, 300 bp for fatty acid synthase, malic enzyme, acetyl-CoA carboxylase and β-actin respectively using the primers described previously by Richards et al. (2003). Polymerase chain amplified product melting temperatures were determined by derivative calculations to determine homogeneity of amplified products. Aliquots of the RT-PCR reactions were loaded onto 1% agarose gels and electrophoresed at 10 V/cm length of gel. Ethidium bromide was used to visualize DNA and orange g was used as the tracking dye. All amplicons were compared to a ladder comprised of known sized DNA standards.

### 2.5. Data analyses

Fluorescence data were used to derive the C(t) or the PCR cycle to threshold which is noted as the first significant deviation in fluorescence from a base line value. The C(t)'s were then transformed to their respective antilogarithmic values. The resultant value was expressed relative to β actin (control gene). Smaller values indicate greater rates of gene expression because greater number of gene copies would result in shorter time periods to the first significant deviation from baseline (Bustin, 2000). Dietary protein levels (12 or 30%) and time (0, 12, 18 and 24 h) were considered as main treatment effects. Significance of certain preplanned comparisons was determined by methods described by Remington and Schork (1970).

## 4. Results

Figs. 1–7 describe the results of switching birds from either a low to a high or high to a low protein diet. Data are presented

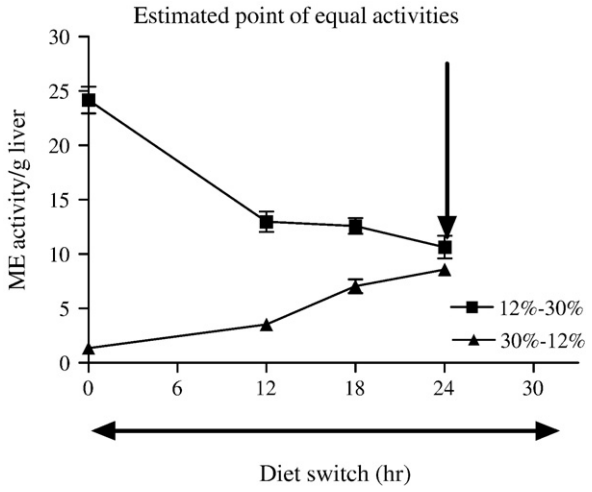


Fig. 2. Malic enzyme activity in chickens switched from a low to a high protein or high to low protein diet. Chickens were initially offered diets containing either 12 or 30% crude protein from 7 to 28 day. Dietary treatments for half the chickens were then reversed and birds were incrementally sampled from 0 to 24 h post reversal. The first number in each legend denotes the dietary crude protein for the 7 to 28-day period and the second number denotes the diet during the reversal period. Values for malic enzyme (ME, EC 1.1.1.40), activity are expressed as µmol of reduced NADP formed per min under standard assay conditions. Data are means of four chickens at each time point. Bars represent SEM.

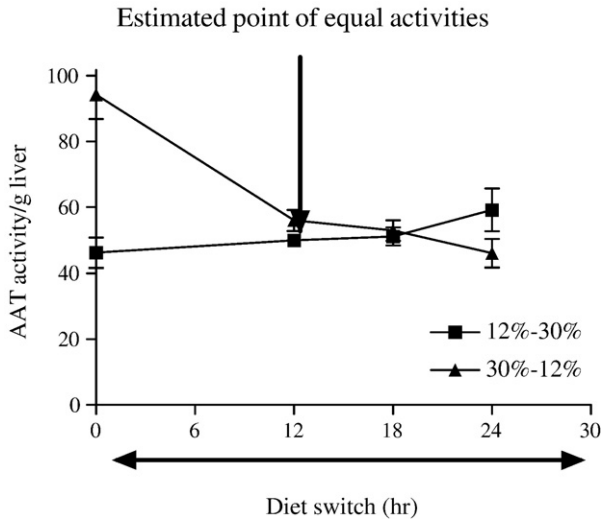


Fig. 3. Aspartate aminotransferase activity in chickens switched from a low to a high protein or high to low protein diet. Chickens were initially offered diets containing either 12 or 30% crude protein from 7 to 28 day. Dietary treatments for half the chickens were then reversed and birds were incrementally sampled from 0 to 24 h post reversal. The first number in each legend denotes the dietary crude protein for the 7 to 28-day period and the second number denotes the diet during the reversal period. Values for aspartate aminotransferase (AAT, EC 2.6.1.1) activity are expressed as μmol of oxidized NAD formed per min under standard assay conditions. Data are means four chickens at each time point. Bars represent SEM.

over the period from 0 to 24 h post switch. Fig. 1 shows that switching birds from either a high (30% protein) or a low (12%) to the opposite protein level resulted in a rapid changes in IVL,

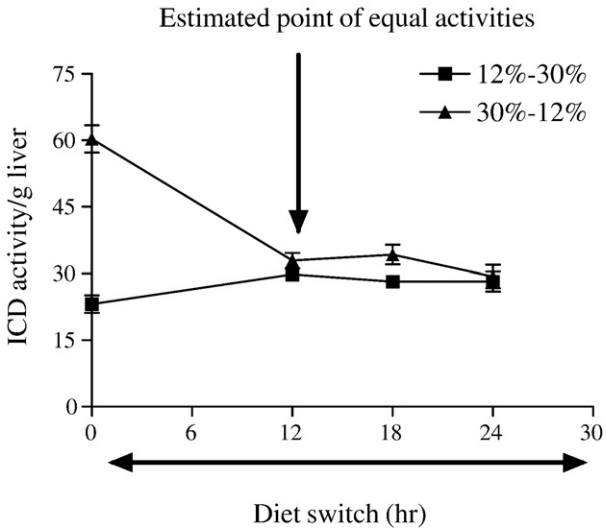


Fig. 4. NADP-linked isocitrate dehydrogenase activity in chickens switched from a low to a high protein or high to low protein diet. Chickens were initially offered diets containing either 12 or 30% crude protein from 7 to 28 day. Dietary treatments for half the chickens were then reversed and birds were incrementally sampled from 0 to 24 h post reversal. The first number in each legend denotes the dietary crude protein for the 7 to 28-day period and the second number denotes the diet during the reversal period. Values for NADP-linked isocitrate dehydrogenase (ICD, EC 1.1.1.42) activity are expressed as μmol of reduced NADP formed per min under standard assay conditions. Data are means of four chickens at each time point. Bars represent SEM.

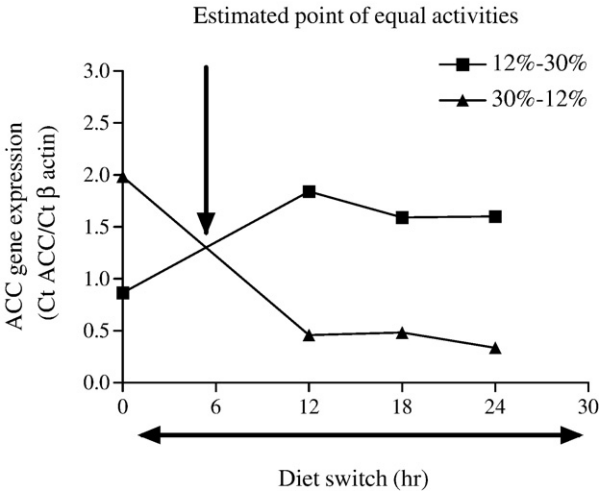


Fig. 5. Acetyl CoA Carboxylase gene expression in chickens switched from a low to a high protein or high to low protein diet. Chickens were initially offered diets containing either 12 or 30% crude protein from 7 to 28 day. Dietary treatments for half the chickens were then reversed and birds were incrementally sampled from 0 to 24 h post reversal. The first number in each legend denotes the dietary crude protein for the 7 to 28-day period and the second number denotes the diet during the reversal period. Data are means of four chickens at each time point. Bars represent SEM.

essentially completed by 12 h post change. Furthermore, regression analysis confirmed that both of these switchbacks resulted in predicted equal rates of IVL slightly before the 12 h point of the switchbacks. In fairness, it should be noted that the rate of decrease (switching from 12 to 30% protein) far exceeded the rate of increase (switching from 30 to 12%).

Fig. 2 summarizes the effect of the two dietary reversals on malic enzyme activity. As noted with lipogenesis, the rate of

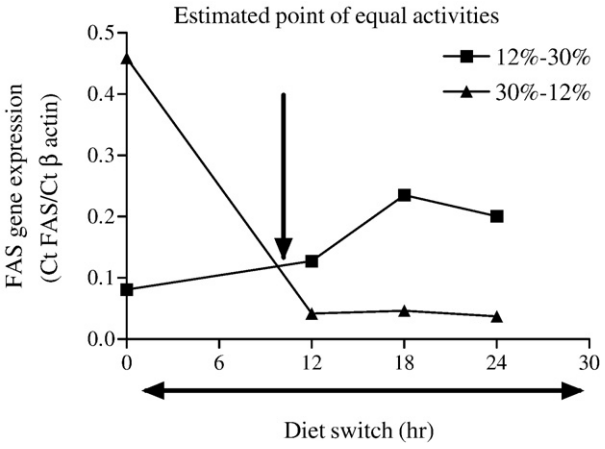


Fig. 6. Fatty acid synthase gene expression in chickens switched from a low to a high protein or high to low protein diet. Chickens were initially offered diets containing either 12 or 30% crude protein from 7 to 28 day. Dietary treatments for half the chickens were then reversed and birds were incrementally sampled from 0 to 24 h post reversal. The first number in each legend denotes the dietary crude protein for the 7 to 28-day period and the second number denotes the diet during the reversal period. Data are means of four chickens at each time point. Bars represent SEM.

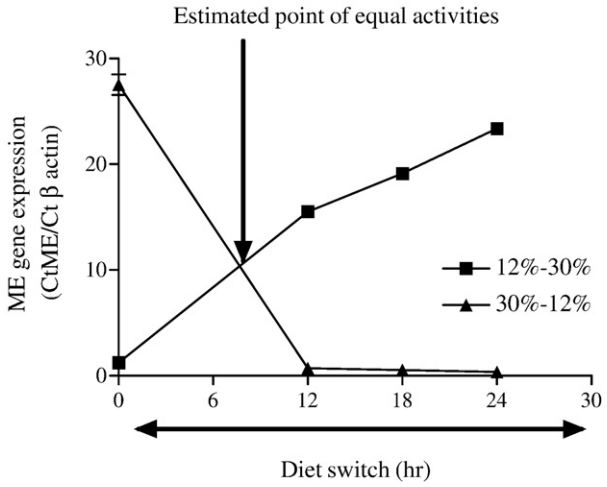


Fig. 7. Malic enzyme gene expression in chickens switched from a low to a high protein or high to low protein diet. Chickens were initially offered diets containing either 12 or 30% crude protein from 7 to 28 day. Dietary treatments for half the chickens were then reversed and birds were incrementally sampled from 0 to 24 h post reversal. The first number in each legend denotes the dietary crude protein for the 7 to 28-day period and the second number denotes the diet during the reversal period. Data are means of four chickens at each time point. Bars represent SEM.

decrease in activity accompanying the switch from 12 to 30% protein was greater than the increased noted when birds were switched from 30 to 12%. In contrast, the estimated point of equal activities was predicted as requiring 24 h as opposed to the much shorter time increment for lipogenesis. Fig. 3, not unexpectedly, demonstrates very little change in AAT activity resulting from the 12–30% switch and a decreases ($P<0.05$) in activity resulting from the 30–12% switch. Again, the apparent estimated point of equal activities in both switch groups was noted at 12 h. Fig. 4 indicates that ICD activity responses were very nearly identical to those noted with AAT.

Figs. 5, 6, and 7 summarize estimates of the expression of certain genes involved in de novo lipid synthesis in birds. Switching from the 30 to the 12% protein diet (30–12%; Fig. 5) significantly decreased ($P<0.01$) the average C(t) for ACC one day after the switch. Equilibrium was reached at 12 h post reversal. Likewise, the opposite switch (12%–30%) significantly increased ($P<0.01$) the average C(t) with equilibrium being reached, again, 12 h after the switch. The regression lines describing both adaptations crossed at less than one day (~6 h) following the switch, indicating equal rates of ACC gene expression for both treatments at this time period. Fig. 6 illustrates adaptations in FAS elicited by the protein reversals. As with ACC the 30%–12% switch significantly decreased ($P<0.01$) the average C(t) while the 12–30% switch significantly increased ($P<0.01$) the average C(t). Both reversals resulted in equilibrium substantially before one day on the reversal diets. The regression lines describing both adaptations again crossed at less than one day (~6 h) following the switch and, again, indicated equal rates of gene expression. The expression of the ME gene was influenced in manner similar to both ACC and FAS (Fig. 7) with an adaptation pattern somewhat like that of ACC.

## 5. Discussion

The data concerning the effects of either a high or low protein diet on IVL in 28-day old birds are consistent with our previous findings. Previous findings from our laboratory have shown that one set of controls (28-day observations) may be sufficient to establish comparisons over at least a five-day period. We have also previously reported that switching birds from either a high or low protein diet to the opposite dietary protein regimen produced rapid changes in metabolism. We have noticed certain general observations concerning the effects of dietary protein on avian metabolism that were confirmed in the present study and add to our previous studies. For example, the present experiment demonstrated that increasing dietary protein would also decrease in vitro lipogenesis as well as malic enzyme activity. Furthermore, work from our group has rather convincingly shown that dietary protein per se influences avian lipid metabolism (Rosebrough and Steele, 1985a,b, 1986; Rosebrough et al., 1986, 1988, 1989, 1990, 2007). In these studies, we formulated diets containing similar amounts of carbohydrate and varied amounts of protein to prove that dietary carbohydrate availability was not the sole determinant in regulating lipogenesis in birds. In this respect, the present experiment supports our previous findings concerning the role of dietary protein per se in regulating avian lipid metabolism.

A recent study (Rosebrough, 2000) showed that changing from a high to a low-protein diet or from a fasted to a fed state rapidly increased lipogenesis in a fashion similar to the meal-feeding response seen in rodents. Ma et al. (1990) reported that feeding fasted chickens stimulated malic enzyme gene transcription in as little as 1.5 h following refeeding.

The purposes of this experiment were twofold 1) to further study the use of a one-step RT-PCR in a real time mode to determine gene expression in birds and 2) to extend our studies on the relationship of dietary protein status and lipid metabolism. We admit that separation of the RT and PCR steps make possible the generation of cDNAs that can be stored stable for extended time periods. Generation of cDNA pools must be balanced against the increase in time required for separate RT-PCR reactions and the possibility of contamination caused by additional pipetting steps. We feel that the greatest advantage in the one-step RT-PCR is the lack of a need for technically difficult cloning steps required for more traditional methods of measuring transcription. In fairness, we admit that older techniques such as Southern Blot analysis will provide information on transcript size as well quality. On the other hand, one of us (Richards and Poch, 2002) has shown that RT-PCR can be coupled with capillary zone electrophoresis to provide estimates of transcript size and quality that are analogous to those provided by Southern Blot analyses. Estimates of transcription are standardized by expressing estimates to that of a control gene not affected by the treatment of interest.

It is appropriate to review prior findings to ascertain some of the genetic mechanisms involved in adaptations to dietary changes. Goodridge et al. (1986) reported that feeding increased and starvation decreased malic enzyme and fatty acid synthase by altering gene transcription in birds. It was interesting to note that increased transcription and mRNA stability were responsible for the increase in malic enzyme seen in restricted vs. ad

libitum fed birds (Richards et al., 2003). In contrast, this same feeding regimen only increased transcription of the fatty acid synthase gene. Furthermore, Goodridge (1987) reported that feeding caused a rapid increase in fatty acid synthase gene transcription prior to any increase in enzyme activity. A much later work by Goodridge et al. (1996) further described the relationship between fasting-refeeding and stimulation of the transcription of the avian gene for malic enzyme.

Semenkovich et al. (1993) reported that glucose availability could control fatty acid synthase mRNA levels without having any effect on transcription initiation. This group seemed to indicate that mRNA stability could be affected by glucose availability. This observation could very easily explain changes in malic enzyme activity and the lack of noted changes in the expression of the genes for malic enzyme, fatty acid synthase or acetyl CoA carboxylase in rodents. The above report may not necessarily explain divergence between gene transcription and enzyme activity in the present study, as carbohydrate intakes among the dietary treatments were nearly equal. The disconnection between gene expression and enzyme activity has been noted in studies on glycogen synthase (Nur et al., 1995). This study revealed that starvation did not change glycogen synthase mRNA but did decrease enzyme activity and that fraction of mRNA associated with polyribosomes. Some of the early work by Goodridge et al. (1986) alluded to the possibility that both increased transcription and mRNA stability were responsible for the increase in malic enzyme mRNA in fasted–refed ducklings.

Lagarrigue et al. (2000) illustrated that mRNA levels of genes coding for certain lipogenic enzymes (acetyl-CoA carboxylase and fatty acid synthase) were not affected by selection for feed intake in spite of consistently larger abdominal fat pads, suggesting an uncoupling between adipogenesis and genes controlling rate-limiting steps of lipogenesis. Previous suggestions had been made that certain lipogenic enzyme activities were regulated at transcriptional steps (Goldman et al., 1985; Goodridge et al., 1986, 1996; Goodridge, 1987) and, by inference, lipogenesis. The very early work of Carlson and Kim (1974) showed that reversible phosphorylation of acetyl CoA carboxylase dramatically changed fatty acid synthesis. Our work has shown that incubation of liver explants in the presence of either isoproterenol or cAMP decreased in vitro lipogenesis 80%, suggesting that rapid changes in lipogenic rates were under the control of phosphorylation-dephosphorylation steps. It is our hypothesis that in vitro lipogenesis is an in situ measurement of, at the very least, acetyl CoA carboxylase activity. A more liberal interpretation would be an in situ measurement of the acetyl CoA carboxylase-fatty acid synthase couple. Hesketh et al. (1998) reported that although nutrition could alter the amount of enzyme protein by transcriptional, post-transcriptional or translational events, increasing evidence indicated that the latter two events were more likely candidates for nutritional control. Previous work by Hillgartner et al. (1996) offered a similar set of observations.

In previous work, we have shown that feeding diets containing crude protein levels from 12 to 21% will dramatically decrease in vitro lipogenesis without changing the expression of genes coding for enzymes involved in lipogenesis (Rosebrough et al., 2002). In contrast, an increase in crude protein to 30% will further decrease lipogenesis in addition to decreasing the expression of genes coding for lipogenic enzymes. Rapid changes in dietary protein will change lipogenesis and expression of lipogenic enzyme genes. The data from these experiments suggest the possibility that a combination of gene expression, mRNA stability and post-transcriptional events interact to regulate lipogenesis in the chicken.

The enzyme activities measured in this study suggest that isocitrate dehydrogenase functions in both lipid and protein metabolism by providing both a residual capacity for the production of reducing equivalents during a period of decreased malic enzyme activity and a coreactant for transamination. In addition, competition may exist between acetyl CoA carboxylase and the aconitase-isocitrate dehydrogenase pathway for limited cytoplasmic citrate. Thus, the requirement for α ketoglutarate as a co-reactant for transamination (AAT) activity) during increased protein intake would depress citrate levels to a point where activation of acetyl CoA carboxylase would not be expected to occur. Clark et al. (1979) reported that avian acetyl CoA carboxylase was particularly sensitive to citrate levels. The latter report seems to offer some support for the role of high-protein diets as regulators of lipogenesis via citrate availability.

In summation, we have shown that real time RT-PCR can be a useful tool in studying genetic adaptations to acute dietary challenges. Using this technique, we found a very rapid change in malic enzyme gene expression following a switch from a low to a high protein level and vice versa. In contrast, expression changes for both acetyl CoA carboxylase and fatty acid synthase, although significant under the same regimens, were not as pronounced. It should be noted, however, that changes in gene expression preceded a change in either metabolic activity or enzyme activity. It is also probable that post-transcriptional events regulate changes in the pathway regulated by their activities.

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